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ALFALFA SEED PRODUCTION; POLLINATION STUDIES.

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INTRODUCTION.

For a number of years past it has been a conspicuous fact that in sections where alfalfa seed is grown commercially the yield varies greatly from season to season. Particularly striking examples of this variation in yield have occurred in the Milk River Valley of Montana, where in some seasons yields of 10 to 12 bushels per acre have been obtained, while in other years the crop was almost a complete failure. It has been generally supposed that the visit of certain insects to the flowers is absolutely necessary in order to effect pollination. In accordance with this belief, some have held that small crops of alfalfa seed were due to an unsatisfactory number of pollinating insects, while others have suggested that thrips or other destructive agencies might be accountable.

In view of the importance of the matter to alfalfa seed growers, investigations of this subject were undertaken, beginning with the season of 1906. These investigations have been conducted during subsequent seasons at various stations and have resulted in the accumulation of a mass of data which throw new light on the subject. Incidentally they have revealed the fact that the problem is much more complex than had been anticipated, and there is need of much further work, especially in the careful correlation of climatic data, as well as the abundance of insects, with the seed yields from season to season. The facts herein set forth substantiate the previous belief in the importance of insect visitors, but also show that, under certain climatic conditions, automatic self-pollination of the flower takes place. The amount of self-pollination varies from season to season and with individual plants. Whether self-pollination is sufficient to produce satisfactory seed yields is still a matter of doubt, but the observations at Chinook, Mont., indicate that at that locality this is the most probable explanation.

Note.—This bulletin deals with the biological problems concerned in the pollination and fecundation of the alfalfa flower. It is intended primarily for technical agronomists and botanists.

Observations at the same place also indicate that the factors or conditions which favor seed production vary during the season, as shown by the distribution of pods on mature plants. For instance, in the latter part of August, 1910, a great many plants could be found on which the earliest racemes to develop in the spring, located at the base of the plant, produced large numbers of pods. A little higher on the plant most of the flower stalks were almost or entirely bare of pods. Still higher on the stem there were a number of large, well-filled clusters of pods, indicating that for a period of two weeks or more preceding August 20 a very large proportion of the flowers had developed pods. Near the tip of the stems nearly all of the flowers fell off, leaving the stem almost bare of pods. It is probable that this variation in seed production at different periods during the season was due, directly or indirectly, to climatic conditions.

At Arlington farm, Virginia, it has frequently been observed that a large proportion of the pods fail to set, even when the flowers have been artificially tripped. While this is especially true of the flowers of the first crop of alfalfa, it seems to be due more to adverse climatic conditions than to the vigor of the plants.

PREVIOUS INVESTIGATIONS OF THE STRUCTURE AND POLLINATION OF THE ALFALFA FLOWER.

According to Urban, the peculiar structure of the alfalfa flower by which it trips, or explodes, when visited by certain insects was known in the time of Linnæus. The first explanation of the process of explosion is apparently that of De Candolle,[1] in 1832. De Candolle states that the explosion of the flower takes place when a certain stage of its maturity is reached.

Hildebrand,[2] in 1866, gives a brief general account of the structure of the alfalfa flower, comparing it with both Indigofera and Cytisus. He clearly recognizes that the peculiar mechanism of the flower is an adaptation for pollination by insects, but states that inclosed flowers finally trip in the course of their development without the help of insects. Apparently he considers that fertilization may also take place in untripped flowers, as the pollen may fall on the stigma. His observations were made in Germany.

In November, 1865, Henslow[3] presented a paper before the Linnæan Society of London, which, however, was not published until 1867. Henslow studied carefully the structure of the alfalfa flower with a view of locating the explosive force. This he attributed to the elasticity of the stamineal tube, but he was uncertain whether the

[1] Candolle, A. P. de. Physiologie Végétale, t. 2, Paris, 1832, p. 548.

[2] Hildebrand, F. Ueber die Vorrichtungen an einigen Blüthen zur Befruchtung durch Insektenhülfe. Botanische Zeitung, Jahrg. 24, No. 10, p. 75, 1866.

[3] Henslow, George. Note on the structure of Medicago sativa, as apparently affording facilities for the intercrossing of distinct flowers. Journal, Linnæan Society, Botany, v. 9, p. 327-329, 1867.

curvature is due to the contraction of the cells on the upper side or the distension of those on the convex side. After the explosion of the flower he states that the tube can not be straightened to its original position without causing a transverse fracture. No similar elasticity was found in the free filament or in the pistil, but the tendency of the keel to open laterally was noted. Henslow also observed honeybees gathering nectar from alfalfa flowers, but in no instance observed by him was the bee able to trip the flower. He also mentions that he did not see bumblebees visiting the flowers. These observations were made in England.

In the same year Delpino described the structure and mechanism of the alfalfa flower. He apparently considered the explosive force due to the irritability of the stamineal tube. Hildebrand [1] criticizes this conclusion and points out that the explosion is due to the tension of the upper filaments in the stamineal tube. He agrees, however, that, after tripping, insects are barred from reaching the nectary.

Urban,[2] in 1873, refers to some of the preceding literature and gives a detailed description of the corolla and of the explosive mechanism. According to his observations, only bees bring about pollination, although butterflies are frequent visitors. In rare cases untripped flowers were found to form pods and seeds. Shortly after the flower has been tripped the opening to the nectary is closed by the drooping of the edges of the standard.

In the same year Müller [3] gave an extended description of the alfalfa flower, together with excellent figures, in which the whole mechanism is clearly explained. The elastic tension of the stamineal column is mainly in the upper stamens, as can be determined by dividing the upper ones from the lower. The former then show much greater curvature. Müller gives a considerable list of insects, including the honeybee and numerous butterflies, which he had observed sucking nectar from the flowers, but states that he never succeeded in seeing the explosion of the flower actually performed by insects, though he watched for it frequently. He also states that self-pollination in untripped flowers does occur, citing Hildebrand's work as confirmatory. Müller also calls attention to certain imperfections of the mechanism of the flower, namely, that nectar secretion continues to take place after the flower is exploded, thus continuing to attract insects without obtaining any additional benefit, and, second, that bees and butterflies can obtain the nectar by inserting the proboscis on one side of the untripped flower, which under no circumstances results in tripping.

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[1] Hildebrand, F. H. G. Federigo Delpino's Beobachtungen über die Bestäubungsvorrichtungen bei den Phanerogamen. Botanische Zeitung, Jahrg. 25, No. 36, p. 283, 1867.

[2] Urban, I. Prodromus einer Monographie der Gattung Medicago L. Verhandlungen, Botanischer Verein, Provinz Brandenburg, Jahrg. 15, p. 13–16, 1873.

[3] Müller, Hermann. Die Befruchtung der Blumen durch Insekten und die gegenseitigen Anpassungen beider. Leipzig, 1873, 478 p., 152 fig.

Henslow[1] in discussing self-fertility in *Medicago sativa* wrote as follows:

> This plant, when protected, yielded seeds, as compared with unprotected, in the ratio of 101:77. Hence it is highly self-fertile, though specially modified, in having "irritable" stamens, for cross-fertilization.

This note of Henslow has been cited by later writers, but it is really an erroneous abstract from Darwin's discussion of *Medicago lupulina*. Darwin[2] writes as follows:

> *Medicago lupulina* (Leguminosæ). On account of the danger of losing the seeds, I was forced to gather the pods before they were quite ripe; 150 flower-heads on plants visited by bees yielded pods weighing 101 grains; while 150 heads on protected plants yielded pods weighing 77 grains. The inequality would probably have been greater if the mature seeds could have been all safely collected and compared.

As Henslow's paper is primarily a review of Darwin's book, it is clear from the two quotations that Henslow erroneously wrote "sativa" in place of "lupulina." This is rendered the more certain as Henslow in his earlier paper on *Medicago sativa* had referred to Darwin's work in a footnote, where the data are properly stated to apply to *Medicago lupulina*.

In 1895 appeared a paper by Burkill,[3] who reviews the principal contributions to this subject by previous writers and adds important new observations and experiments. He verifies the conclusions of earlier investigators that the explosive action of the flower depends on the uppermost stamens of the stamineal tube. Burkill obtained no pods in a considerable number of flowers covered with nets to prevent insect visits, for which phenomenon he presents an interesting explanation:

> Pollen is shed in the bud and lies round the stamens and stigma in a little lens-shaped space made by the carina. . . . No seeds are set in the unexploded flower in spite of the pollen in contact with the stigma. This is explained by the fact that the stigma does not become receptive until rubbed or until its cells are injured in some manner. My proof is, I think, conclusive. Firstly, the stigma appears not to be moist, but when rubbed on glass leaves a sticky mark. Secondly, I have caused flowers to set seed though unexploded, (1) by pinching the stigma through the keel, (2) by perforating the keel and rubbing the stigma with a stiff paint brush, and (3) by cutting off the tip of the keel and rubbing the stigma with a stiff paint brush. An insect visitor exploding the flower will injure the stigmatic papillæ and bring about fertilization.

Burkill gives a list of 31 insects which he observed visiting alfalfa flowers in and near Cambridge, England. In no case did he see a butterfly causing the flower to trip, but on one hot afternoon he

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[1] Henslow, George. On the self-fertilization of plants. Transactions, Linnæan Society, London, Botany, s. 2, v. 1, pt. 6, p. 361, 1879.

[2] Darwin, Charles. The Effects of Cross and Self Fertilization in the Vegetable Kingdom. New York, 1877, p. 368.

[3] Burkill, I. H. On the fertilization of some species of Medicago L. in England Proceedings, Cambridge Philosophical Society, v. 8, pt. 3, p. 142-147, 1894.

watched a bumblebee tripping the flowers in great numbers and on two occasions observed honeybees doing the same thing.

In artificially tripped flowers Burkill found that 12 out of 34 tripped set seed; 50 flowers from which the standard had been removed were artificially tripped and none set seed. The impact on the standard, Burkill believes, ruptures the stigma sufficiently to insure fertilization in about one-third of the cases. Burkill's interesting data on the tripping of alfalfa flowers when vertical force is applied to the tip of the keel are quoted in full on page 27 of this paper.

Hunter[1] conducted observations on the relation of the number of seeds per pod in alfalfa as correlated with the proximity of domestic honeybees. He evidently assumes that honeybees are capable of pollinating the flowers, but he does not record any observations of his own on this point. Pods were compared from two fields, one within half a mile of a large apiary, the other 25 miles distant from any domestic bees, none of which were observed in the latter field. Of pods taken half a mile from a large apiary, 87 contained 482 seeds, or 5.58 per pod; 80 pods taken 25 miles distant from any colony of domestic bees produced 268 seeds, or 3.35 per pod.

Kirchner,[2] after pointing out that the data on the self-fertilization of alfalfa are contradictory, gives results of his own experiments at Hohenheim, Germany. Of exposed clusters of blossoms, 54 on two plants with 432 blossoms produced, August 23, 208 pods, which, though they were not perfectly ripe, showed that they contained 636 well-developed seeds. On the other hand, 21 covered clusters of blossoms on the same plants with 166 blossoms produced only 2 pods with 3 seeds. He concludes that alfalfa flowers are self-sterile, and suggests that Henslow's results were due to some experimental error.

Westgate,[3] in 1906, presented a brief review of the work of Henslow, Urban, Burkill, and Kirchner, calling attention to the disagreements in the results of different investigators and pointing out the need of further studies.

Fruwirth[4] found that inclosed plants occasionally formed a few pods at Vienna, Austria.

Roberts and Freeman[5] have recorded results of alfalfa pollination experiments at the Kansas Agricultural Experiment Station. Great

[1] Hunter, S. J. Alfalfa, grasshoppers, bees: their relationship. University of Kansas, Department of Entomology, contribution 65, p. 84, 1899.

[2] Kirchner, O. Über die Wirkung der Selbstbestäubung bei den Papilionaceen. Naturwissenschaftliche Zeitschrift fur Land- und Forstwirtschaft, Jahrg. 3, Heft 1, p. 9–10, 1905.

[3] Westgate, J. M. A method of breeding a strain of alfalfa from a single individual. American Breeders' Association, Proceedings, v. 2, p. 65–67, 1906.

[4] Fruwirth, Carl. Die Züchtung der Landwirtschaftlichen Kulturpflanzen. Bd. 3, Berlin, 1906, p. 189.

[5] Roberts, H. F., and Freeman, G. F. Alfalfa breeding: materials and methods. Kansas Agricultural Experiment Station, Bulletin 151, p. 79–109, 14 fig., 1908.

differences were observed among individual plants as regards seed production. Of seven plants which showed marked differences in this respect, five were classified as "strong" and two as "weak." When these plants were inclosed in screens to exclude pollinating insects the same tendencies remained evident, two of the plants producing pods and seeds in much greater numbers than the others.

In a second series of plants inclosed in screens and self-pollinated by hand the percentage of pods to flowers pollinated varied on different plants from 5.5 per cent to 65.4 per cent, and in one exceptional instance 115 per cent. In this last case some flowers evidently formed pods without hand pollination. A single plant was inclosed in a wire cage to exclude insects. On one half the stems the flowers were self-pollinated by hand and produced 97 pods containing 118 seeds. The other half, not hand pollinated, produced 37 pods containing 59 seeds.

In another experiment the investigators inclosed one half of each of five plants in a screen cage, leaving the other half exposed to natural conditions of pollination. The flowers inclosed in the cage were self-pollinated by hand; those outside the cage were naturally pollinated, but not necessarily cross-pollinated by insects, as assumed. The results they obtained are shown in Table I. A remarkable feature is the extraordinarily large proportion of sterile pods recorded.

TABLE I.—*Results of natural and of artificial pollination of alfalfa, at Manhattan, Kans., by Roberts and Freeman.*

Plant.	Method of pollination.	Weight of stems (grams).	Number of stems.	Number of pods.	Pods producing seeds.		Number of seeds.		
					Number.	Per cent.	Produced.	Average per pod.	Per 10 grams weight of plant.
No. 29	Insects	49.87	11	255	30	11.76	61	2.03	12.2
	Hand	35.63	9	272	12	4.41	14	1.17	3.9
No. 38	Insects	103.88	12	327	91	27.82	164	1.80	15.7
	Hand	114.00	18	279	164	58.77	236	1.44	20.7
No. 97	Insects	28.50	8	239	65	27.19	67	1.03	23.5
	Hand	37.00	20	608	103	16.94	128	1.24	34.6
No. 98	Insects	85.50	11	449	228	50.78	451	1.96	52.7
	Hand	64.13	8	779	57	7.30	70	1.22	10.9
No. 109	Insects	14.00	6	198	67	33.83	96	1.43	68.5
	Hand	14.00	8	311	180	57.87	239	1.32	170.7
Summary	Insects	281.75	48	1,468	481	32.76	839	1.74	29.7
	Hand	264.76	63	2,249	516	22.49	687	1.33	25.9

Brand and Westgate[1] give a brief discussion of the relation of insects to the production of alfalfa seed. These authors assert that "insect visitors are essential to the proper pollination of the alfalfa flower." They state that bumblebees are the most efficient of all insects in tripping the flowers and hence bring about pollination.

[1] Brand, C. J., and Westgate, J. M. Alfalfa in cultivated rows for seed production in semiarid regions. U. S. Department of Agriculture, Bureau of Plant Industry, Circular 24, 23 p., 3 fig., 1909.

Honeybees are not nearly so effective as bumblebees, but should not be underrated in this connection, while bees of the genera Andrena and Megachile and various butterflies are also valuable agents in pollinating alfalfa flowers.

Results are also given showing the seed production of plants whose flowers were artificially tripped in comparison with untreated plants. At Arlington farm, Virginia, artificial tripping resulted in an increased production of 25.5 per cent, while at Chico, Cal., an increase of 129 per cent of pods was obtained.

Piper,[1] in a report of the American Breeders' Association committee on forage crops relating to the breeding of alfalfa, gives an epitome of the answers of members to various subjects of inquiry, five of which relate to pollination. The answers are diverse, some of them based on experiment and careful observations and others more or less expressions of opinion.

Westgate [2] records that he observed over 500 visits of honeybees to alfalfa flowers, the flower being tripped in but one case.

Wildermuth [3] records with some doubt that he has seen the butterfly of the alfalfa caterpillar (*Eurymus eurytheme*) trip alfalfa flowers. In a personal interview he states that he observed five or six individuals trip the flowers during one season, but has not seen it since, though he has frequently watched the butterflies. This butterfly is very common on alfalfa throughout the Western States.

It will be noted from the brief reviews given that investigators have differed as to their conclusions on several points in connection with the pollination of the alfalfa flower. The most important questions that affect the problem of seed yield left in doubt are the following:

1. To what extent are the flowers self-fertile?
2. Is cross-pollination more effective than self-pollination?
3. Do alfalfa flowers trip automatically?
4. Do untripped flowers form pods and seeds?
5. Is the rupturing of the stigma essential to its becoming receptive?
6. To what extent do honeybees trip alfalfa flowers?

In the investigations reported in this paper will be found abundant data which go far to clear up the discrepancies in previous work.

STRUCTURE OF THE ALFALFA FLOWER.

The structure of the alfalfa flower has been described and illustrated in detail by Hermann Müller and other writers. The most interesting feature is the explosive apparatus which functions to facilitate pollination and under proper conditions to favor cross-pollination. The

[1] Piper, C. V. Alfalfa and its improvement by breeding. American Breeders' Association, Report, v. 5, 1908/09, p. 94–115 1909.

[2] Westgate, J. M. Methods of breeding alfalfa by selection. American Breeders' Association, Report, v. 5, 1908/09, p. 147, 1909.

[3] Wildermuth, V. L. The alfalfa caterpillar. U. S. Department of Agriculture, Bureau of Entomology, Circular 133, p. 1, 1911.

essential parts of the mechanism (fig. 1) are the tension of the stamineal tube, which is held from contracting by two opposite restraining lateral processes on the inside of the keel. These processes are really

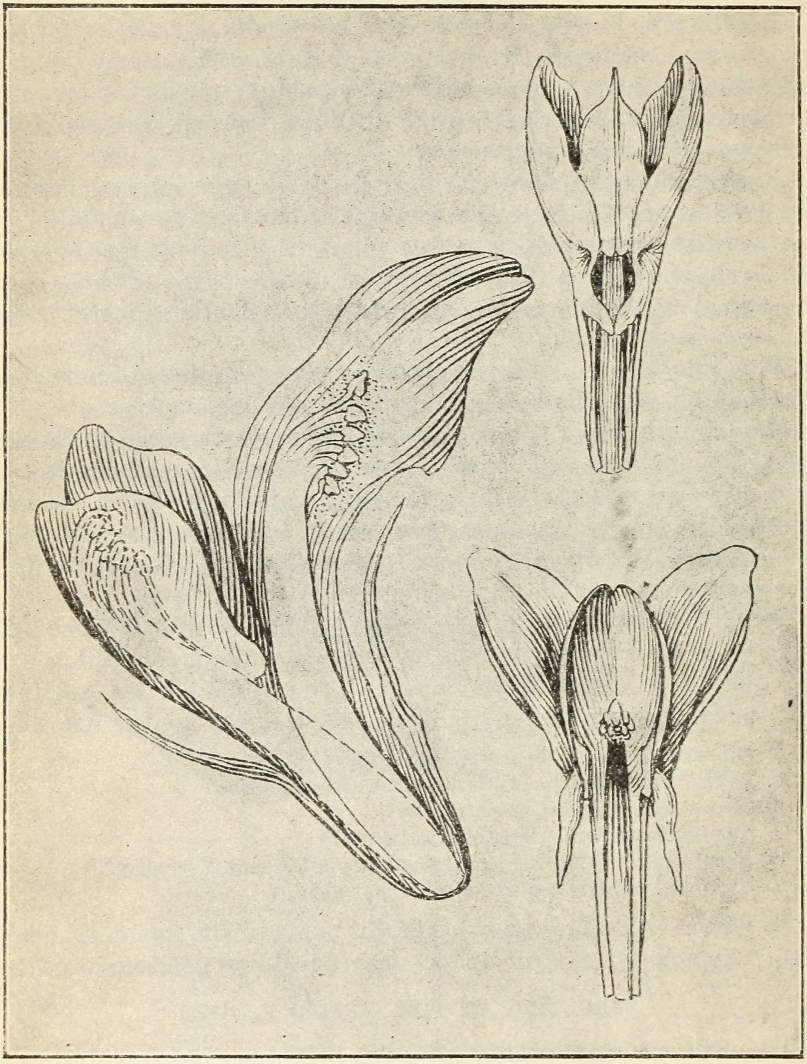

FIG. 1.—Alfalfa flower (much enlarged). The left-hand figure shows an optical section of the flower, indicating the position of the stamineal column before and after tripping. The upper right-hand figure gives a view from above of an untripped flower with the calyx and standard removed; the lower right-hand figure, the same after tripping.

invaginations, on the outside occurring as depressions. Each of the wing petals is provided with two fingerlike processes, one extending forward and the other backward. The anterior process of each wing fits into the depression on the same side of the keel, and the two wings

thus serve to strengthen the keel. Contrary to Müller's statement, both of the wings can, by exercising great care, be removed without tripping the flower, thus showing that their function is purely secondary. The posterior processes of the wing meet on top of the stamineal column. They can have but little, if any, effect in confining the column in position, as Henslow supposed, for the reason above stated, namely, that their removal is not necessarily followed by explosion. The keel is not purely passive, but its basal tissues are under a lateral tension which tends to pull it open, as Henslow first observed. This tension is restrained by the pressure of the stamineal tube against the two internal knobs. If both the apex and the base of the stamineal column are severed by a razor, so that pressure is removed from the keel, the latter will open automatically. If the edges of the keel are again brought together, they open as soon as the restraining force is removed. In an uninjured flower a very slight separation of the edges of the keel, and consequently of the restraining knob, will release the tense stamineal column. Heavy insects, like bumblebees, may do this by their combined weight and pressure on the tip of the keel, but usually it is accomplished by the insect's proboscis separating ever so slightly the upper posterior edges of the keel. This may be done directly, but more commonly by spreading apart the two posterior processes of the wings and thus indirectly spreading the keel. The terminal part of the keel, notwithstanding the cohesion of the two petals, has little influence to prevent tripping, as, with the inclosed stamineal tube and style, it can be cut off with a razor without releasing the explosive mechanism.

As shown by Henslow, and perhaps earlier by Delpino, the elastic tension lies entirely in the coalesced filaments of the nine anthers and not at all in the style. With care the style can be severed at the base without affecting the tripping movements, as Henslow pointed out.

After tripping, the opening to the nectary is almost closed by the upcurved stamineal tube, but insects continue to visit tripped flowers until the wilting of the petals makes the closure complete.

The occurrence of this explosive mechanism is not unique in the alfalfa flower, but is found in at least 20 other species of Medicago which have been examined in this connection. In yellow or sickle alfalfa the stamineal column is relatively much shorter, but the mechanism is the same. It is also very well developed in *Medicago scutellata, M. rugosa, M. turbinata, M. rigidula, M. ciliaris,* and *M. echinus.* It is less noticeable in some other annual species, because the stamineal column is shorter and not exposed when tripped, as in alfalfa and the species just mentioned. Other genera in which tripping mechanisms occur are Alysicarpus, Trigonella, Indigofera, and Genista.

RELATION OF TRIPPING TO THE DEVELOPMENT OF SEED.

In order to obtain more abundant data in regard to the relation of tripping to the production of pods and seeds, the experiment here described was conducted:

On selected plants, approximately two-thirds of the branches were inclosed in fine-meshed mosquito-bar tents to prevent insects from having access to the flowers. Tarlatan was the material used for the netting. The meshes averaged 25 to the linear inch, thus being from one-half to one-third finer meshed than ordinary mosquito bar, which was thought to be too coarse to exclude the smaller insects which might gain access. The remaining stems of each plant were left outside the tent, where the flowers could develop under natural conditions. On both portions of the plant a number of racemes were marked, each with a tag, and numbered. All unopened flower buds and all wilted or tripped flowers were cut off from each of these racemes, leaving only the fresh, open, and untripped flowers. The flowers on approximately one-half of the marked racemes inside the netting were artificially tripped. This was done usually by means of a slender alfalfa stem or grass stem, pushed down between the keel and the standard. On some of the plants used in this experiment, a separate stem was used for each flower, so as to exclude pollen from other flowers, while on other plants the same stem was used to trip several flowers on the same plant. The flowers were fertilized in all cases, therefore, with pollen from the same flower or with pollen of other flowers on the same plant. The flowers on the remaining racemes inside of the netting tent were allowed to develop without being tripped through the visit of insects or by any artificial agency.

This experiment was carried out at Pullman, Wash., in 1908, 1909, and 1910; at Chico, Cal., in 1908 and 1909; at Arlington farm, Virginia, in 1908; at Chinook and Havre, Mont., in 1909; and at New London, Ohio, in 1912. The summarized results are shown in Table II.

TABLE II.—*Pods and seeds produced by alfalfa flowers on the same plants, free and protected from insects.*

A.—OUTSIDE NETTING: FLOWERS DEVELOPED UNDER NATURAL CONDITIONS.

Year.	Place.	Number of plants.	Total number of flowers.	Number of pods.	Total number of seeds.	Flowers bearing pods.	Average number of seeds per pod.
						Per cent.	
1908	Pullman	7	633	155	426	24.47	2.74
	Chico	15	3,474	321	681	9.24	2.12
	Arlington	1	80	35	31	43.75	.88
1909	Pullman	15	1,468	480	1,730	32.69	3.60
	Chinook	10	944	143	320	15.14	2.23
	Havre	9	366	51	.....	13.93	.....
	Chico	8	1,589	228	.....	14.34	.....
1910	Pullman	6	228	30	38	13.15	1.26
1912	New London	6	157	56	105	35.66	1.80
Total		77	8,939	1,499	3,331	.....	.....
Average		.....	.....	.....	.....	16.76	2.22

TABLE II.—*Pods and seeds produced by alfalfa flowers on the same plants, free and protected from insects*—Continued.

B.—INSIDE NETTING: FLOWERS NOT TRIPPED EITHER ARTIFICIALLY OR BY INSECTS.

Year.	Place.	Number of plants.	Total number of flowers.	Number of pods.	Total number of seeds.	Flowers bearing pods.	Average number of seeds per pod.
						Per cent.	
1908	Pullman	7	651	25	29	3.84	1.16
	Chico	15	4,116	157	230	3.81	1.46
	Arlington	1	37				
1909	Pullman	15	1,500	131	357	8.73	2.72
	Chinook	10	1,186	138	282	11.63	2.04
	Havre	9	535	30		5.60	
	Chico	8	832	15		1.80	
1910	Pullman	6	314	12	22	3.82	1.83
1912	New London	6	169	11	11	6.50	1.00
Total		77	9,340	519	931		
Average						5.55	1.78

C.—INSIDE NETTING: FLOWERS ARTIFICIALLY TRIPPED.

1908	Pullman	7	576	148	205	25.67	1.38
	Chico	15	4,229	1,086	1,908	25.67	1.75
	Arlington	1	22	7	9	31.81	1.30
1909	Pullman	15	1,379	599	1,783	43.43	2.97
	Chinook	10	830	370	681	44.57	1.84
	Havre	9	337	104		30.86	
	Chico	8	1,250	345		27.60	
1910	Pullman	6	296	75	103	25.33	1.37
1912	New London	6	155	50	91	32.25	1.80
Total		77	9,074	2,784	4,780		
Average						30.68	1.72

In Table II there are no data to indicate what percentage of flowers actually tripped by insects or other natural agencies form pods. Data on this point were secured by observations at Pullman, Wash., in 1908. Flowers which had been tripped naturally were marked by putting a drop of insoluble drawing ink on the calyx of each. The racemes were then inclosed in netting to prevent other insect visits. The results are shown in Table III.

TABLE III.—*Pods and seeds produced from alfalfa flowers tripped under natural conditions.*

Raceme.	Number of flowers.			Number of pods developed from—		Total number of seeds developed.	
	In raceme.	Tripped.	Not tripped when inclosed.	Tripped flowers.	Flowers not tripped when inclosed.	From tripped flowers.	From flowers not tripped when inclosed.
No. 1	11	4	7	2		2	
No. 2	11	8	3	5		33	
No. 3	16	11	5	8		55	
No. 4	8	6	2	3		2	
No. 5	17	11	6	7	1	11	1
No. 6	10	5	5	4		14	
No. 7	18	10	8	2		2	
No. 8	9	9		8		33	
No. 9	11	6	5	5		11	
No. 10	13	8	5	4		3	
No. 11	15	8	7	6		25	
No. 12	11	7	4		2		2
Total	150	93	57	54	3	191	3

Tripped flowers bearing pods	per cent	58.06
Flowers not tripped when inclosed bearing pods	do	5.26
Average number of seeds per pod from tripped flowers		3.53
Average number of seeds per pod from flowers not tripped when inclosed		1.00

The abundant data presented in Table II permit the following conclusions:

(1) Flowers not tripped either artificially or by insects may produce pods. The percentage of pods to flowers under these conditions varies from 11.63 to 0, the average for 77 plants being 5.55.

(2) Flowers artificially tripped produce pods in percentages ranging from 25.33 to 44.57, the average for 9,074 flowers on 77 plants being 30.68.

(3) Under natural conditions the percentage of flowers setting pods varies from 9.24 to 43.75. The average percentage of pods from 8,939 flowers on 77 plants is 16.76.

(4) The number of seeds per pod in artificially tripped flowers is usually less than in naturally fertilized flowers, the average number of seeds per pod for 77 plants being 1.72 in the former case and 2.22 in the latter. In caged plants not tripped either artificially or by insects the pods averaged 1.78 seeds each. The larger number of seeds per pod in the exposed portions of the plants is perhaps due to cross-pollination.

RELATION OF INSECTS TO TRIPPING.

To obtain data on the efficiency of insects in tripping alfalfa flowers, observations have been made at Pullman, Wash.; Chinook and Havre, Mont.; Chico, Cal.; and at Arlington farm, Virginia. No attempt was made to secure a list of visiting species, the object being rather to ascertain the relation, if any, of insect visitors to seed formation.

BEES.

Among the commonest insects which visit alfalfa flowers are honey-gathering bees. The data from detailed observations made at Pullman, Wash., and Chinook and Havre, Mont., are shown in Table IV.

TABLE IV.—*Alfalfa flowers tripped by different honey-gathering bees.*

Year.	Species.	Where observed.	Total number of flowers visited.	Flowers tripped.	
				Number.	Per cent.
1909	Apis mellifica	Pullman, Wash	318	1	0.31
1910	do	do	189	3	1.58
1909	do	Chinook, Mont	126	6	4.76
1909	Bombus spp.[1]	Havre, Mont	268	79	29.47
1909	Megachile latimanus	Pullman, Wash	52	47	90.38
1909	do	Chinook, Mont	45	42	93.33

[1] Four species of Bombus were found tripping flowers at Havre, viz, *B. auricomus* Robertson, *B. separatus* Cresson, *B. bifarius* Cresson, *B. borealis* Kirby.

It will be noted that the leaf-cutting bee (*Megachile latimanus* Say) is by far the most efficient, tripping about nine flowers out of every ten visited. Bumblebees are decidedly inferior to Megachile, tripping

only about 30 per cent of the flowers visited. No attempt was made to secure records of different species of Bombus, but there is certainly considerable difference in their ability to trip the flowers. The larger bumblebees are clumsy insects and at Chico have been observed to trip with their feet flowers other than the one in which the proboscis was inserted. Honeybees trip but few alfalfa flowers, as previously noted by other observers.

In 1907 a single individual of *Megachile latimanus* was observed to trip 4 flowers in 30 seconds at Pullman, Wash.; another tripped 12 flowers in 70 seconds, and a third tripped 20 flowers in 2 minutes and 15 seconds. These three bees tripped flowers at the rate of 9.2 flowers per minute, or 552 flowers per hour.

The process of tripping is thus described by Evans:

> When *Megachile latimanus* visits an alfalfa flower, it grasps the wings or the keel from below, braces its head up against the standard, and in this way forces the wing and keel petals apart from the standard, so that it can push its head down and reach the honey. As a result, the flower is usually tripped. When this occurs, the pollen is thrown in a miniature cloud that is sometimes visible to the eye. There is abundant opportunity for the pollen to lodge upon the head and other parts of the bee, where portions of it can easily come in contact with the stigma of the next flower that is tripped. Occasionally the proboscis of a bee is caught by the pistil, which after the flower is tripped presses up quite closely to the standard. When thus caught, the bee braces up on all six legs and after one or two vigorous shakes releases itself. Such an accident does not result in any injury to the bee, but merely occasions a short delay. The insect then rubs its proboscis with the two front feet and flies off to gather honey from other flowers.

Honeybees were also carefully observed by McKee at Chico, Cal., in 1909. But few flowers were tripped by these insects, though repeated visits seemed to increase the ease of tripping. Thus, one flower tripped after four visits by honeybees; another, after seven visits. In other cases, however, the flowers did not trip even after seven visits by honeybees.

Short-tongued bees of the genus Andrena have also been observed tripping alfalfa flowers both at Pullman, Wash., and at Arlington farm, Virginia.

BUTTERFLIES.

Various species of butterflies are among the common insect visitors to alfalfa flowers, the most abundant at Pullman, Wash., and Chinook, Mont., being species of Pieris and Eurymus. Several species of butterflies were carefully observed at the two places above mentioned as well as at Chico, Cal., and at Arlington farm, Virginia, but in no case was an individual seen to trip a flower. In all cases the butterfly inserts its proboscis at one side of the flower. Our observations on these insects agree fully with those of Urban.

MOTHS AND OTHER NIGHT-FLYING INSECTS.

Owing to the fact that tripped flowers are sometimes abundant while day-flying insect visitors are scarce, it was suspected that night-flying insects might be a factor. To secure information on this point two series of experiments were undertaken.

One of these experiments was conducted at Pullman, Wash., in 1909. Seven alfalfa plants were inclosed in fine-meshed mosquito netting. Five of the plants were left under the netting during the entire time of the experiment in order to find out what proportion of the flowers become tripped when insects were entirely excluded. Two of the plants were kept inclosed in netting during the daytime, but were uncovered during the night. The results obtained are given in Table V.

TABLE V.—*Alfalfa flowers tripped by night-flying insects at Pullman, Wash., 1909.*

On plants inside of netting.			On same plants outside of netting; open to insects.	
Plant.	Flowers tripped.	Flowers bearing pods.	Plant.	Flowers bearing pods.
	Per cent.	*Per cent.*		*Per cent.*
No. 11	* 0	* 1.16	No. 11	37.31
No. 12	* 0	* 0	No. 12	16.12
No. 14	* 3.92	* 8.57	No. 14	56.60
No. 15	* 3.03	* 2.59	No. 15	20.51
No. 17	* 0	* 1.36	No. 7	35.38
No. 7	† 4.63	† 5.78	No. 10	30.35
No. 10	† 4.60	† 2.51		

* Inclosed in netting during entire experiment. † Inclosed in daytime; open for night-flying insects.

A similar experiment was conducted at Chico, Cal. On May 31, 1909, 400 alfalfa flowers on several different plants were marked and observed until June 2. Table VI shows how many flowers were tripped during the day or night.

TABLE VI.—*Alfalfa flowers tripped during different periods of the day and night at Chico, Cal., 1909.*

Period covered.		Number of flowers tripped.	Period covered.		Number of flowers tripped.
From—	To—		From—	To—	
4 p. m., May 31	7.30 p. m., May 31	22	11 a. m., June 1	2 p. m., June 1	16
7.30 p. m., May 31	5.30 a. m., June 1	0	2 p. m., June 1	4 p. m., June 1	18
5.30 a. m., June 1	10 a. m., June 1	23	4 p. m., June 1	7.30 p. m., June 1	18
10 a. m., June 1	11 a. m., June 1	11	7.30 p. m., June 1	6 a. m., June 2	0

From the evidence presented in the two preceding tables, as well as from the results of observations made at other times and places, it is clear that night-flying insects are at most a small factor in tripping alfalfa flowers.

MISCELLANEOUS INSECTS.

A number of species of small insects visit alfalfa flowers, not for the purpose of getting honey, but to feed upon the pollen and the cellular tissue of the flower. The most common insects of this kind are the thrips. These insects are found in all parts of the United States, and have frequently been abundant in the alfalfa flowers at Arlington farm, Virginia; Chico, Cal.; Pullman, Wash.; and at Chinook and Havre, Mont. At Pullman 1,119 thrips were found on 16 racemes of alfalfa flowers, or an average of 69.9 thrips on the flowers of each raceme. At Havre 48 thrips were found on 13 racemes. These minute insects do not trip the alfalfa flowers.

In conducting the various experiments described in this paper, it has been observed that even when the thrips are present in very large numbers the flowers very rarely develop into pods and seed unless tripped. On the other hand, when the flowers are tripped, a large proportion usually produce pods and seeds, even though the thrips are very abundant. The evidence at hand indicates that the thrips are neither appreciably beneficial nor injurious in their influence upon the development of alfalfa seed.

Another insect commonly found on alfalfa flowers is the tarnished plant bug (*Lygus pratensis*). Blister beetles (*Epicauta puncticollis* Mannerheim) are found on alfalfa in abundance at Pullman, Wash., feeding on the more tender portions, especially the stamens and style. At Brookings, S. Dak., occurs the related *Macrobasis unicolor* Kirby. This beetle, according to R. A. Oakley, does considerable damage to the flowers, but incidentally trips many.

EFFECTS ON SEED SETTING OF VISITS OF INSECTS WITHOUT TRIPPING FLOWERS.

To determine whether or not the visiting of flowers by insects without actual tripping aids in seed setting, observations were made at Chico, Cal., as shown in Table VII. The plants designated as A, B, and C were in full bloom at the time of beginning the experiment, and up to that time were not protected in any way. After counting and tagging the old and young flowers to be observed, the plants were screened with tarlatan netting, with the exception of a portion of plant B, which was left to develop under natural conditions.

The flowers designated as old flowers were the oldest on the plant not tripped at the time the experiment was started, and many of them probably had been visited one or more times by bees or other insects without tripping. That insects had visited the older flowers is merely assumption, but as many bees and other insects were working the alfalfa flowers on the days immediately preceding the starting of the experiment, this seems probable. The flowers designated as young flowers were not yet in bloom when they were screened.

TABLE VII.—*Effects on seed setting of visits of insects without tripping the alfalfa flower at Chico, Cal., 1909.*

Description of flowers.	Plant.	Number of flowers.	Pods set.	
			Number.	Per cent.
Young flowers untripped and protected from insect visitors	A	109	3	2.74
	B	34	0	0
	C	40	1	2.5
Old flowers untripped and protected from further insect visitors	A	311	5	1.6
	B	47	0	0
	C	33	0	0
Young flowers developed under natural conditions	B	52	31	59.61
Old flowers developed under natural conditions	B	70	18	25.71

These results indicate that a small percentage of flowers set pods without insect visitors and that insect visitors that do not trip the flowers have no effect.

EFFECT OF POLLEN FROM DIFFERENT SOURCES.

To determine the relative effects of self-pollination and cross-pollination, a series of experiments was conducted at Pullman, Wash., Chico, Cal., and New London, Ohio. Using the same female parent, pollen was applied to the stigma (*a*) from the same flower, (*b*) from another flower on the same plant, and (*c*) from another plant of the same variety. The results are presented in Table VIII.

TABLE VIII.—*Pods and seeds produced by alfalfa flowers when fertilized by pollen from different sources.*

A.—WHEN POLLINATED FROM THE SAME FLOWER.

Locality.	Year.	Plant No.	Number of flowers.	Number of pods.	Flowers bearing pods.	Number of seeds.	
						Total.	Average per pod.
					Per cent.		
Chico, Cal.	1909	3399	65	17	26.0	.....	.....
Do.	1909	5099	95	34	35.7	.....	.....
Pullman, Wash.	1910	1	43	9	20.9	11	1.2
Do.	1910	3	29	2	6.9	2	1.0
Do.	1910	5	61	42	68.8	55	1.3
Do.	1910	7	50	19	38.0	34	1.8
New London, Ohio.	1912	1	33	1	3.0	0	0
Do.	1912	2	26	16	61.5	24	1.5
Do.	1912	3	13	0	0	0	0
Do.	1912	4	27	13	48.1	26	2.0
Do.	1912	5	32	12	37.5	6	.5
Do.	1912	6	39	0	0	0	0
Total.	.....	.....	513	165	.....	158	.....
Average.	.....	.....	.....	.....	32.1	.....	1.4

TABLE VIII.—*Pods and seeds produced by alfalfa flowers when fertilized by pollen from different sources*—Continued.

B.—WHEN POLLINATED FROM DIFFERENT FLOWERS ON THE SAME PLANT.

Locality.	Year.	Plant No.	Number of flowers.	Number of pods.	Flowers bearing pods.	Number of seeds.	
						Total.	Average per pod.
					Per cent.		
Chico, Cal.	1909	3399	26	4	15.4	.....	.....
Do.	1909	5099	28	12	42.9	.....	.....
Pullman, Wash.	1910	1	34	9	26.5	18	2.0
Do.	1910	3	69	9	13.0	18	2.0
Do.	1910	5	66	33	50.0	90	2.7
Do.	1910	7	59	17	28.8	21	1.2
New London, Ohio.	1912	1	31	1	3.2	0	0
Do.	1912	2	23	19	82.6	63	3.3
Do.	1912	3	15	6	40.0	8	1.3
Do.	1912	4	26	14	53.8	13	.9
Do.	1912	5	28	8	28.5	7	.8
Do.	1912	6	32	2	6.3	0	0
Total.	.....	.....	437	134	.....	238	.....
Average.	.....	.....	.....	.....	30.6	.....	2.02

C.—WHEN POLLINATED FROM FLOWERS OF DIFFERENT PLANTS OF THE SAME VARIETY.

Chico, Cal.	1909	3399	44	31	70.5	.....	.....
Do.	1909	5099	43	15	34.9	.....	.....
Pullman, Wash.	1910	1	38	18	47.4	39	2.2
Do.	1910	3	54	21	38.8	113	5.4
Do.	1910	5	48	24	50.0	49	2.0
Do.	1910	7	51	20	39.6	58	2.9
New London, Ohio.	1912	1	34	10	29.4	13	1.3
Do.	1912	2	25	18	72.0	27	1.5
Do.	1912	3	19	9	47.3	14	1.5
Do.	1912	4	20	16	80.0	47	2.9
Do.	1912	5	35	14	40.0	15	1.1
Do.	1912	6	35	10	28.5	7	.7
Total.	.....	.....	446	206	.....	382	.....
Average.	.....	.....	.....	.....	46.1	.....	2.38

In the first and second sections of Table VIII the most striking fact is the wide variation of the individual plants in their ability to form seed when the flower is self-pollinated or pollinated from another flower of the same plant. There is clearly a great difference between individuals in this respect. In the matter of averages, 513 self-pollinated flowers produced 165 pods, or 32.1 per cent, while 437 flowers, each pollinated from another flower on the same plant, produced 134 pods, or 30.6 per cent. On the same 12 plants, 446 flowers, each cross-pollinated from another plant of the same variety, produced 206 pods, or 46.1 per cent.

From 114 pods of the self-pollinated flowers 158 seeds were produced, an average of 1.4 seeds per pod; 118 pods from the flowers pollinated from another flower on the same plant contained 238 seeds, an average of 2.02 seeds per pod; while 160 pods from the cross-pollinated flowers contained 382 seeds, or 2.38 seeds per pod.

It would appear, therefore, that cross-pollination is more potent than self-pollination, while pollination from another flower on the

same plant gives practically the same results as self-pollination. As the same 12 female parent plants were used in all the experiments, the factor of individual variation is eliminated.

At Chico, Cal., in 1909, flowers on different branches of two selected alfalfa plants were pollinated, (*a*) from the same flower, (*b*) from a different flower on the same plant, (*c*) from a separate plant of the same variety, and (*d*) from a different variety. The female parent in one case was a plant of Peruvian alfalfa, S. P. I. No. 3399; in another, ordinary alfalfa, F. C. I. No. 5099; and in the third, Turkestan alfalfa, S. P. I. No. 18751. The results at Chico and also of similar experiments conducted at Arlington farm in 1908 and 1909 are given in Table IX.

TABLE IX.—*Results of the pollination of alfalfa flowers from different parents.*

CHICO, CAL., 1909.

Female parent.	Male parent.	Number of flowers.	Number of pods.	Flowers bearing pods.	Number of seeds.	
					Total.	Average per pod.
				Per cent.		
3399	Same flower	65	17	26.0		
3399	Another flower on same plant.	26	4	15.4		
3399	Another plant of same variety.	44	31	70.5		
3399	19822, Turkey	70	48	68.5		
3399	12694, Provence	76	46	60.5		
3399	....do	16	8	50.0		
3399	18823, Arabia	38	28	73.7		
3399	991, Turkestan	47	27	57.5		
3399	18827, Utah	24	11	45.8		
5099	Same flower	95	34	35.7		
5099	Another flower on same plant.	28	12	42.9		
5099	Another plant of same variety.	43	15	34.9		
5099	Grimm	68	44	64.7		
18751	Same flower	112	5	4.5		
18751	Another plant of same variety.	50	23	46.0		
18751	12694, Provence	16	8	50.0		

ARLINGTON FARM, VIRGINIA.

F. C. I. 1, M. sativa	Same flower	125	62	49.6	108	1.7
Do	F. C. I. 28, M. sativa (Kan.).	14	9	64.3	39	4.3
Do	F. C. I. 34, Grimm	16	15	93.8	50	3.3
Do	F. C. I. 2072, M. falcata	5	3	60.00	17	5.6
Do	S. P. I. 20571, sand lucerne	38	27	71.00	126	4.7
Do	F. C. I. 152, Kansas variegated.	8	6	75.00	31	5.1
Do	S. P. I. 19534, M. falcata	4	4	100.00	16	4.0
F. C. I. 18, M. sativa	Same flower	267	172	64.4	354	2.0
Do	F. C. I. 2072, M. falcata	73	51	69.9	259	5.0

The results indicate that cross-pollination is usually much more efficient than self-pollination, whether the latter is by the same flower or by another flower of the same plant. The efficiency of cross-pollination is about the same, regardless of whether the pollen-producing parent is the same or a different variety.

RELATION OF THE NUMBER OF FLOWERS PER RACEME TO THE NUMBER OF PODS FORMED.

In Table III there is some slight evidence to indicate that racemes with many flowers produce proportionately fewer pods than racemes with few flowers. This matter was further investigated by McKee at Chico, Cal., in 1909, and his results are shown in Table X. According to these results it would appear that few-flowered racemes produce proportionately twice as many pods as many-flowered racemes. While this factor is evidently one to be taken into consideration, it could hardly modify materially the results shown in Table II, owing to the very large number of flowers counted in these experiments.

These data are the combined readings from 15 different plants. Exactly analogous conclusions are shown, however, by tabulating the results of each individual plant.

TABLE X.—*Effect of the number of alfalfa flowers per raceme on the percentage of pods set.*

Number of flowers per raceme.	Number of racemes.	Number of flowers.	Number of pods.	Number of pods per raceme.	Number of seeds.		Flowers setting pods.
					Total.	Average per pod.	
							Per cent.
2 to 6	153	707	317	2.07	553	1.7	44.8
7 to 11	138	1,212	464	3.36	806	1.7	38.2
12 to 16	50	669	205	4.1	401	1.9	30.6
17 to 26	17	344	68	4.0	96	1.4	19.7

AUTOMATIC TRIPPING.

The term "automatic tripping" is used when an alfalfa flower becomes tripped without the aid of insects or any other external body. This phenomenon was first actually observed at Chinook, Mont., in 1909, but was suspected from observations of the previous season at the same place. In 1909 two of the plants inclosed in netting produced pods on racemes from which insects had been excluded. To obtain further facts in regard to the process, all wilted and all unopened flowers were removed from a number of the racemes under the netting, leaving only opened, untripped flowers, which were closely observed during the following days. In the course of a day or two several of the flowers had become tripped. On one of these two plants the keel petals were partially separating in some of the flowers. While these flowers were being examined one flower was seen in the process of tripping. The pistil and stamens snapped up vigorously against the standard, scattering the pollen around. No object had come in contact with any portion of the flower.

The calyx of each tripped flower was marked with carbon ink as soon as it was detected. Those which did not trip were watched until the corolla had wilted or the flower had fallen. The number of

flowers that were tripped and the pods that developed from tripped and untripped flowers are shown in Table XI.

TABLE XI.—*Pods and seeds from automatically tripped alfalfa flowers at Chinook, Mont., 1909.*

Plant.	Raceme No.	Number of flowers.		Flowers tripped.	Number of pods.			Flowers bearing pods.
		Total.	Tripped.		Total.	From tripped flowers.	From untripped flowers.	
				Per cent.				*Per cent.*
No. 3	34	12	9	75.00	7	7	0	58.33
No. 3	35	11	3	27.27	3	3	0	27.27
No. 3	36	7	2	28.57	0	0	0	0
No. 3	37	11	7	63.63	5	5	0	45.45
No. 3	38	10	9	90.00	5	5	0	50.00
No. 3	39	6	3	50.00	1	1	0	16.66
Total		57	33		21	21	0	
Average				57.88				36.84
No. 8	30	14	5	35.71	3	3	0	21.42
No. 8	31	6	2	33.33	0	0	0	0
No. 8	32	20	14	70.00	8	8	0	40.00
No. 8	33	11	2	18.18	0	0	0	0
No. 8	34	13	13	100.00	5	5	0	38.46
Total		64	36		16	16	0	
Average				56.25				25.00

Flowers tripped on plants 3 and 8.....per cent.. 57.02
Flowers producing seed on plants 3 and 8.....do..... 30.57
Tripped flowers producing seed on plants 3 and 8.....do..... 53.62
Untripped flowers producing seeds on plants 3 and 8.....do..... 0

At Arlington farm, Virginia, in 1909, an entire alfalfa plant was inclosed in a screen of tarlatan about the time the first flowers came into bloom. Ten days or two weeks later the plant was observed to be in full bloom. When the screen was removed it was noticed that the flowers seemed larger than those on plants that had not been screened. Seventeen racemes were tagged to show the number of flowers on each, all unopened flowers being removed. The screen had been removed only two or three minutes when a snapping or clicking sound was heard. On close observation it was found that some of the flowers had become tripped. The sound of the column striking the standard was quite distinct, but even with very close watching no single flower was actually seen in the act of tripping. No insects visited the flowers of this plant, and the only way in which the flowers could be tripped would be automatic. The day was clear, very warm, and with no breeze stirring. The screen was removed about 11 o'clock in the morning and for not more than 15 minutes. No actual count of the flowers tripped in the manner just described was made. At first the flowers did not trip very fast, but as the plant remained longer in the sunshine the trippings became more frequent. At times three or four would be heard almost simultaneously. The

flowers that tripped first were on the outside of the plant. The screen was replaced, so that insects had no access to the flowers, nor was the screen again removed until the seed was mature.

It was estimated that more than one-half of the flowers counted on each raceme thus became self-tripped before the screen was replaced. None were artificially tripped, as extreme care was taken. The results are presented in Table XII.

No flowers already tripped were noticed when the screen was first removed. Apparently the tripping was induced when the screen was removed by the increased transpiration from the turgid flowers. Certainly no insect agency was involved. The total number of pods produced was 23.7 per cent of the total number of flowers counted and tagged.

TABLE XII.—*Results obtained with a single alfalfa plant screened from insects and exposed for 15 minutes on a bright, warm day when in full bloom, thus bringing about the automatic tripping of its flowers.*

Raceme.	Number of flowers.	Number of pods.	Flowers bearing pods.	Number of seeds.	
				Total.	Average per pod.
			Per cent.		
No. 1	26	10	38.4	13	1.3
No. 2	16	3	18.7	6	2.0
No. 3	12	3	25.0	7	2.3
No. 4	17	8	47.0	10	1.2
No. 5	20	5	25.0	14	2.8
No. 6	18	4	22.2	8	2.0
No. 7	12	1	8.3	3	3.0
No. 8	22	3	13.6	6	2.0
No. 9	14	2	14.2	4	2.0
No. 10	14	1	7.1	2	2.0
No. 11	16	2	12.5	4	2.0
No. 12	18	2	11.1	5	2.5
No. 13	17	6	35.2	12	2.0
No. 14	17	4	23.5	10	2.5
No. 15	24	5	20.8	10	2.0
No. 16	12	4	33.3	7	1.7
No. 17	20	7	35.0	12	1.7
Total	295	70	.....	133	.....
Average	.....	.....	23.7	.....	1.9

In 1910 an experiment was conducted at Pullman, Wash., for the purpose of determining what proportion of flowers became self-tripped. Accordingly, nine alfalfa plants, which were producing more pods and seeds than most of the alfalfa plants in the experimental plats, were inclosed in netting tents and were closely observed for several days.

The tents in which the plants were inclosed were carefully covered with fine-mesh mosquito bar, so that no openings were left for honey-gathering insects to gain access to the flowers. The tents were made large enough and pains were taken so that no flowers which were being watched were in such a position that they could brush against the sides or top of the tent.

After the flowers from which insects were to be excluded had been inclosed in the netting tents, they were examined every day or every second day until all of the flowers had become entirely wilted. Whenever any flower was found tripped, the calyx was marked with a mixture of carbon black and water in order that the pod which might develop from the tripped flower could be distinguished from any pod that might develop from a flower that had not been observed to be tripped. The unmarked flowers were observed until they wilted, so it is certain that none of them became tripped.

Table XIII shows the number of flowers that became tripped and also the number of pods and seeds that developed from tripped and untripped flowers.

TABLE XIII.—*Pods and seeds from self-tripped alfalfa flowers at Pullman, Wash., 1910*

Plant.	Number of flowers.			Number of pods from marked flowers.	Number of mature seeds from marked pods.	Number of pods from flowers not marked.	Mature seeds from pods not marked.
	Total.	Tripped and marked.	Not tripped and not marked.				
No. 8	128	9	119	1	0	0	0
No. 9	104	17	87	12	18	4	4
No. 10	59	2	57	1	0	1	0
No. 11	100	15	85	4	3	0	0
No. 12	62	4	58	0	0	0	0
No. 13	91	1	90	1	1	0	0
No. 18	79	3	76	2	4	0	0
No. 19	71	8	63	0	0	0	0
No. 20	81	1	80	0	0	0	0
Total	775	60	715	21	26	5	4

Flowers tripped	per cent	7.74
Tripped flowers producing pods	do	35.00
Flowers not tripped producing pods	do	.69
Average number of mature seeds per pod from tripped flowers		1.23
Average number of mature seeds per pod which developed from flowers not observed to be tripped		.80

Table XIII shows that 7.7 per cent of the flowers observed on the nine plants became tripped. In several of the flowers the keel was observed gradually to open, and later the flowers were found to be tripped. As the tents were made so that honey-gathering insects did not have access to the flowers and as care was taken to prevent any other object from coming in contact with the flowers, it seems clear that the tripping which occurred was automatic.

Five of the 775 flowers observed produced pods, when no evidence that these flowers had been tripped could be found. It is possible that three of these flowers may have become tripped without being observed or that the carbon which was placed on the calyx may have been removed. However, two flowers were found in which the pod was developing and the tip of the young pod was protruding through

the tip of the keel while the flower remained untripped. This proves that a pod may develop from a flower without the flower having been tripped. The further evidence obtained in this investigation, however, indicates that it is only in rare instances that untripped alfalfa flowers produce seed.

In some seasons the alfalfa plants in the fields about Chinook, Mont., produce seed in abundance, though honey-gathering insects are present in only very small numbers. In other seasons most of the flowers fall off the plants without producing pods and seed, and only light seed crops are harvested. On August 22, 1910, it was found that practically all flowers that had been open for more than a few hours had been tripped and that during a period of 10 days or 2 weeks prior to this time a very large proportion of the flowers had developed into pods. A typical raceme was found to have on it 20 flowers which had been opened. Of this number 15 had become tripped. The 5 flowers not tripped were newly opened, all being located near the top of the raceme. For several days the weather had been bright and warm, though not excessively hot. On this date flowers just beginning to open were inclosed in netting tents and were watched in order to determine what was causing the flowers to become tripped. At this time, however, the weather became much colder, a heavy frost occurring on the night of August 24. After the weather became colder comparatively few flowers were tripped or produced pods. Of a total number of 390 marked flowers inside netting tents, only 1 became tripped; of 333 marked flowers on plants not inclosed in netting, only 3 became tripped. Practically all of the flowers that opened after August 22 fell off the plants without producing pods. Since honey-gathering insects were comparatively rare in the alfalfa fields during the warm weather prior to August 22, it seems evident that a large proportion of the flowers were becoming automatically tripped and that when the weather became colder conditions were no longer favorable for the flowers to become tripped and produce seed.

From the evidence presented, there can be no question that automatic tripping does occur in alfalfa flowers. It also indicates that in rare cases pods form without the flower becoming tripped.

The evidence also shows that atmospheric or climatic conditions greatly affect automatic tripping, so that it is not improbable that this factor alone accounts for a great variation in seed production during different seasons at the same place.

In 1913 it was found at Arlington farm that alfalfa flowers could readily be tripped by focusing sunlight upon them with a burning glass. The tripping takes place without any evident wilting.

At Brookings, S. Dak., in 1913, R. A. Oakley observed that when the flowers in the shade near the ground were carefully raised into

the hot sunshine automatic tripping took place quickly and could easily be observed. These observations remove any possible doubt as to the movement being automatic. It will take place, however, only when the sun's heat is intense. It now seems clear that automatic tripping is induced mainly by hot sunshine, though it is not proved that flowers continually in the sunshine will be tripped to the same extent as those alternately in the shade and exposed.

POLLINATION IN RELATION TO THE RUPTURE OF THE STIGMATIC CELLS.

Burkill's conclusion that the stigma is not susceptible of pollination until the stigmatic cells have been ruptured has already been mentioned. To test this matter further, the following investigations were conducted by J. M. Westgate in the greenhouse at Washington, D. C., by W. J. Morse at Arlington farm, Virginia, and by M. W. Evans at Pullman, Wash. Two methods were used. In the first, the standard was removed and the flower then tripped. The results of the experiment with this method at the three places mentioned are shown in Table XIV.

TABLE XIV.—*The setting of alfalfa pods by flowers tripped after removing the standard.*

Observer.	Place.	Year.	Number of plants.	Number of flowers tripped.	Number of pods formed.	Flowers bearing pods.	Number of seeds.	
							Total.	Average per pod.
						Per cent.		
Westgate	Washington, D. C.	1908	18	468	74	15.8	.....	.....
Morse	Arlington farm	1908	5	123	28	22.8	37	1.3
Do	do	1909	5	23	10	43.4	16	1.6
Evans	Pullman, Wash	1909	.....	113	14	12.4	.....	.....

These results show clearly that the mechanical effect of the stigma striking the standard is not necessary to insure fertilization. Morse also tripped flowers, allowing the stigma to strike the standard. In these experiments, 76 flowers in 1908 produced 18 pods, or 23.7 per cent, and 42 flowers in 1909 produced 22 pods, or 52.4 per cent. These figures are but slightly larger than where the standard was removed. When the stigma was allowed to strike a piece of wood used in tripping, 21 flowers in 1908 produced 3 pods, and 12 flowers produced 4 pods—percentages 14.3 and 33.3, respectively. Though the numbers of flowers used in the last test were small, the results do not indicate that any additional benefit was secured.

The second method was to remove the standard and then sever the keel at the base with a razor. The column thus retained its position in the keel unchanged after tripping. The results of the experiment with this method are shown in Table XV.

TABLE XV.—*The setting of alfalfa pods by flowers when the standard was removed and the keel severed at base.*

Observer.	Place.	Year.	Number of plants.	Number of flowers tripped.	Number of pods formed.	Flowers bearing pods.	Number of seeds.	
							Total.	Average per pod.
						Per cent.		
Westgate	Washington, D. C.	1908	13	246	52	21.1	.....	.....
Morse	Arlington farm	1908	10	120	19	15.9	28	1.4
Do	do	1909 [1]	6	22	12	54.0	23	1.9
Do	do	1909 [2]	8	27	11	40.8	19	1.7
Evans	Pullman, Wash.	1909	.....	76	13	17.1	.....	.....

[1] Plants screened. [2] Plants not screened.

The proportion of pods to flowers is in fair agreement with those tripped otherwise. It would seem, therefore, that no importance can be attached to the keel as a passive agent of friction when the column retracts.

ARTIFICIAL AGENCIES EFFECTIVE IN TRIPPING.

Any force giving sufficient pressure on the keel, either laterally or vertically, will result in tripping the flower. This may be accomplished by means of the fingers or even by the hand in grasping an entire cluster or bunch of clusters. Tripping may also be accomplished by means of a stick, straw, or pencil point inserted into the flower and then separating the posterior processes of the wing petals. In order to determine the relative effect of these various means, an experiment was carried out in which tripping was accomplished in the following ways: (1) Each flower tripped with a small piece of wood, (2) with a pencil point, and (3) rolled between the fingers.

The data obtained (Table XVI) show little difference in the effects produced by these various methods of tripping.

TABLE XVI.—*Effect of tripping alfalfa flowers by different mechanical methods, at Arlington farm, Virginia.*

Method of tripping.	Year.	Plant.	Number of flowers.	Number of pods.	Flowers bearing pods.	Number of seeds.	
						Total.	Average per pod.
					Per cent.		
With a small piece of wood.	1908	F. C. I. No. 18	21	3	14.2	7	2.3
	1909	F. C. I. No. 1	12	4	33.3	5	1.2
With a pencil point	1908	F. C. I. No. 18	76	18	23.7	33	1.8
	1909	F. C. I. No. 1	42	22	52.3	38	1.7
By rolling between the thumb and fingers.	1908	F. C. I. No. 18	75	40	53.3	71	1.7
	1908	F. C. I. No. 1	22	7	31.8	9	1.3
	1909	do	43	21	48.8	42	2.0

EFFECT OF AGE OF FLOWERS UPON SUSCEPTIBILITY TO FERTILIZATION.

An experiment by Westgate was performed in one of the greenhouses of the United States Department of Agriculture at Washington, D. C., for the purpose of determining whether the age of the flower affects its susceptibility to fertilization. A single plant of Peruvian alfalfa (F. C. I. No. 60) was used in this experiment. A number of young, prime, and old flowers were artificially tripped. For the purpose of comparison, a number of flowers at each stage of development were marked, but not tripped. Table XVII shows the percentage of flowers which produced pods under each method of treatment.

TABLE XVII.—*Pod setting in relation to age of alfalfa flowers when tripped.*

Age.	Number of flowers tripped.	Number of pods formed.	Flowers bearing pods.	Flowers not tripped.	Number of pods formed.	Flowers bearing pods.
			Per cent.			*Per cent.*
Young flowers	15	7	46.66	18	0	0
Prime flowers	34	17	50.00	38	0	0
Old flowers	81	40	49.38	89	3	3.37

A similar experiment, designed to show at what stages of its development an alfalfa flower may become fertilized and also to throw some light on the question as to how long it may remain capable of fertilization, was conducted at Pullman, Wash., by Evans in 1909. All opened and wilted flowers were removed from a number of racemes on five different plants inclosed in netting tents. On the following day all unopened buds on these racemes were removed, leaving only those flowers which had opened during the preceding 30 hours. As the experiment was carried out in September, when the weather was comparatively cool, the flowers remained fresh and open for a longer period than would have been the case in warmer weather. A number of the flowers on these racemes were tripped each day up to the end of seven days, when the tips of some of the petals were beginning to wilt. The experiment was discontinued at this time because the supply of flowers was exhausted. The number of the flowers that were tripped and the percentage of tripped flowers which produced pods are shown in Table XVIII.

TABLE XVIII.—*Results obtained at Pullman, Wash., in tripping alfalfa flowers at different intervals after blooming.*

Time from opening of flower until tripped.	Number of flowers.	Number of pods formed.	Flowers bearing pods.
			Per cent.
1 day	103	28	27.18
2 days	69	18	26.08
3 days	96	36	37.50
4 days	64	21	32.81
5 days	79	19	24.05
6 days	53	28	52.83
7 days	64	16	25.00

A number of flowers were left untripped on these plants. Less than 5 per cent of the untripped flowers produced pods.

The results of both experiments, as tabulated, show that there is no definite relation between the age of the flowers when tripped and the proportion of pods developed. Clearly there is no diminution in the ability of the flowers to become fertilized as long as the flowers remain open.

FORCE NECESSARY FOR TRIPPING.

Burkill's results in measuring the force necessary to trip alfalfa flowers are thus reported:

> That the separation of the basal processes is the legitimate and almost only natural method of exploding the flower is obvious from the following consideration. By means of a fine wire hung onto the alæ, weights to a known extent were suspended from them. In September, 1892, flowers obtained near Poulton (Gloucestershire) were found to explode with an average weight of 1.68 grams (maximum and minimum, 2.37 and 0.93). Now, an insect visiting the flower rests its weight on the points whence these weights were hung. The worker of Apis I find to weigh about 0.096 and *Bombus hortorum* (large specimens) 0.199 grams. The mere weight of these two insects is therefore quite insufficient to explode the flower. Moreover, the pedicel of the flower bends under a weight insufficient to explode the flower, so that in these experiments I found it necessary always to fix the flower by a wire hooked into the standard; and, again, the hive bee so settles as to hold the parts of the flowers together with its feet.
>
> By the same method of experiment I discovered that the flower is not always in the same degree of explosiveness; the hotter the weather the more explosive is the flower. In cold weather the flower frequently remains unexploded for eight or nine days, after which it withers, but in hot, sunny weather I found three days to be the maximum duration, for explosion is brought about often within 24 hours from the opening of the bud. We must remember in this connection that *M. sativa* is of Persian origin and has only traversed Europe northward by slow degrees.
>
> Shaking by the wind can not explode the flowers. Pieces of paper with a surface of 18½ and 22 square inches were tied to stalks of this plant in order to give more power to the wind, but no effect was observable from the shaking it produced.[1]

Table XIX gives the results of experiments carried out by Westgate with an apparatus similar to that used by Burkill. His results confirm those of Burkill in showing that the force required is much greater than the mere weight of bumblebees or other insects which trip alfalfa flowers. They also show clearly that the force required diminishes as the flowers become older. There is also a considerable range of variation in the weight required to trip different flowers of approximately the same age.

[1] Burkill, I. H. On the fertilization of some species of Medicago L. in England. Proceedings, Cambridge Philosophical Society, v. 8, pt. 3, p. 146, 1894.

TABLE XIX.—*Weight (in grams) necessary to trip alfalfa flowers on different plants at different stages of maturity.*

AT ALMA, NEBR.[1]

Plant.	Young flowers.	Prime flowers.	Old flowers.
F. C. I. 138	2.14	1.49	0.68
F. C. I. 139	3.89	3.37	3.37
M. 1221	6.42	3.56	2.60
M. 1222	3.37	2.54	1.30
F. C. I. 142	7.12	3.69	2.14
Average	5.18	2.92	2.07

AT ARLINGTON FARM, VIRGINIA.[2]

F. C. I. 99	6.57	2.22	1.68
S. P. I. 17992	2.07	1.49	.33
S. P. I. 21232	4.79	3.04	.45
Average	4.21	2.33	.77

[1] Experiment performed August 4, 1908. Temperature 98° to 101° F.; humidity very low; bright sun. Number of flowers used in experiment as follows: Young, 16; prime, 22; old, 21.
[2] Experiment performed July 2, 1908; used one flower at each stage of maturity.

Observations made at different times and places indicate that wind or rain do not ordinarily cause alfalfa flowers to become tripped. Even when blowing with high velocity the wind sways the plants back and forth usually without causing the racemes to strike against adjoining plants. At Chinook, Mont., during a high gale accompanied by a rainstorm, which lasted for a few minutes, a small proportion of a number of flowers that had been marked was tripped. The number of flowers which become tripped through the influence of wind or rain, however, in ordinary seasons is evidently small.

EFFECT OF PARTIAL SHADE.

In conducting the experiments described in this paper it has been observed that on the different plants used those flowers which have been inclosed in the covering made by a single thickness of mosquito bar remain in bloom longer and that the petals seem to be larger than on those flowers which were not inclosed. As it seemed possible that the effect of the slight shade or the breaking of the force of the wind might also influence the development of pods and seed, an experiment was performed at Pullman, Wash., in 1908, to obtain some information in regard to this point.

Several plants were selected, and a portion of each plant was inclosed in a tent made of netting (tarlatan); the remaining portion of each plant was not inclosed, but was protected on three sides and partially from above by one thickness of netting, which was between the plant and the sun and also between the plant and the prevailing winds, yet did not prevent the access of bees and other insects. The results of this experiment are shown in Table XX, in which the results secured on unprotected plants at the same time and place are also presented for the purpose of comparison.

TABLE XX.—*Pods and seeds formed on alfalfa plants at Pullman, Wash., in 1908 under different conditions as regards shade and insect visitation.*

Plant.	Conditions.	Number of flowers.	Number of pods developed.	Flowers bearing pods.	Number of seeds.	
					Total.	Average per pod.
	Flowers open to insects, sun, and wind	869	207	*Per cent.* 23.8	574	2.77
No. 03	Shaded by one thickness of netting, insects not excluded	160	35	21.8	53	1.51
No. 03-A.		71	21	29.5	71	3.38
No. 41		112	31	27.6	88	2.83
No. 89		99	22	22.2	43	1.95
No. 89-A.		71	18	25.3	41	2.27
	Total	513	127	.....	296	.....
	Average	.....	.....	24.7	.....	2.33
No. 03	Entirely inclosed in mosquito netting	141	14	9.9	19	1.35
No. 03-A.		99	7	7.0	19	2.71
No. 41		132	4	3.0	5	1.25
No. 89		105	2	1.9	3	1.50
No. 89-A.		43	0	0	0	0
	Total	520	27	.....	46	.....
	Average	.....	.....	5.1	.....	1.70

As shown in Table XX, 23.8 per cent of the flowers which developed under natural conditions produced pods. The average number of seeds per pod was 2.77. On the stems where the flowers were screened from sun and wind by one thickness of mosquito netting but which were open to the visits of honey-gathering insects, 24.7 per cent of the flowers produced pods, which contained an average number of 2.33 seeds each. On the stems which were entirely inclosed by one thickness of mosquito netting, thus excluding insects, 5.1 per cent of the flowers produced pods, which contained an average number of 1.7 seeds each.

In this experiment the proportion of flowers producing pods and the number of seeds per pod were practically the same when the flowers were screened from sun and wind by one thickness of mosquito netting, which did not exclude insects, as when the flowers developed under natural conditions. It is evident from these results that the shade produced by a covering of one thickness of mosquito netting had in this case no appreciable effect on the setting of pods.

PRACTICAL ASPECTS OF TRIPPING.

The recognition of the efficiency of tripping in the seed setting of alfalfa makes it possible to secure inbred seed whenever cross-pollination is not desired. This can be accomplished by isolating the plants to any degree that is necessary and then tripping, using such artificial means as are called for by the extensiveness of the operations. The observed increase of the seed crop thus obtained indicates the possibility of adopting this method on an extended scale to secure alfalfa-seed crops, especially where the plants are grown in cultivated

rows. It may be practicable to utilize some simple type of machine that will artificially trip alfalfa flowers and thus increase the seed crop. This subject is at present under investigation. Another possibility lies in propagating such bees as are effective pollinators. It might, indeed, be profitable to introduce into the United States the bees that are most effective in the native land of alfalfa.

TRIPPING IN RELATION TO SEED SETTING IN ANNUAL MEDICAGOS.

To determine the relation of tripping by insects to seed setting in the annual medicagos, a number of the species were covered by cages so made as to exclude all but very minute insects and left through the flowering and fruiting period. The species which at Chico, Cal., formed pods readily and apparently as well as in the open are shown in Table XXI.

TABLE XXI.—*Species of annual medicagos readily forming pods under cover of cages.*

Plant designation.	Species.	Plant designation.	Species.
S. P. I. No. 10725	Medicago orbicularis.	S. P. I. No. 17783	Medicago tuberculata.
S. P. I. No. 19433	M. hispida confinis.	S. P. I. No. 26077	M. scutellata.
S. P. I. No. 22649	M. hispida denticulata.	S. P. I. No. 28790	M. tuberculata aculeata.
S. P. I. No. 19449	M. turbinata.	S. P. I. No. 9743	M. muricata.
S. P. I. No. 19442	M. rugosa.		

Whether the flowers in any of these species set seed without tripping can not be stated positively, but so far as could be determined they became tripped before setting seed, and apparently the tripping was automatic. However, this point could not be determined definitely in all cases. The flowers in some of the species have a very short stamineal column. This makes it difficult to observe the tripping process as readily as in species with a long stamineal column.

In two species (S. P. I. No. 30111, *Medicago ciliaris*, and S. P. I. No. 16874, *Medicago echinus*) pods did not form in as large numbers inside the cages as outside, but even with these a number of pods formed in the cages.

To determine whether artificial tripping is beneficial in *Medicago echinus*, flowers of this species were worked as follows:

Six racemes, containing 28 flowers, were artificially tripped, resulting in three pods forming.

Four racemes, containing 21 flowers, were left untripped as a check, and these set one pod.

The number of racemes rather than the number of flowers should be used as a basis of comparison, as not all of the flowers ever set pods.

In *Medicago echinus* there are five to seven flowers in a cluster, but only one or two burs form from this number, even though all the flowers in a cluster are tripped.

GENERAL CONCLUSIONS.

The numerous researches of previous investigators on the pollination of the alfalfa flower have resulted in somewhat divergent conclusions. In but few cases has any attempt been made to determine the relation of pollination to the resultant crop of seed.

The opinion has prevailed that insect pollinizers are of vital importance and that in the absence of these in adequate numbers the resultant seed crop is necessarily small.

It has, however, been generally recognized that climatic conditions are important, as practically all the commercial seed is raised in regions having a semiarid climate, at least during the time the seed crop is made.

Alfalfa flowers remain fully susceptible to pollination from the time of opening until the petals wither. Pollination is ordinarily effected when the elastic stamineal column has become "tripped." No evidence was found to favor Burkill's theory that tripping effects the rupture of the stigmatic cells and that this is an important factor in fertilization. Flowers tripped in various ways to prevent any stimulation or rupturing of the stigma by contact set pods equally as well as those tripped naturally.

Flowers tripped artificially, and therefore self-pollinated, set pods freely. In one series of experiments on 77 plants at 7 different places, 9,074 flowers set 2,784 pods when artificially tripped (a percentage of 30.68), while 8,939 flowers on the same plants exposed to natural conditions set 1,499 pods (16.76 per cent). The pods from artificially tripped flowers contained an average of 1.72 seeds each, while those from naturally tripped flowers averaged 2.22 seeds each.

Pollination from a different flower on the same plant is no more effective than self-pollination, but pollen from another plant increases both the proportion of pods set and the number of seeds per pod. It makes but little difference whether the pollen parent be the same or a different variety.

Tripping in alfalfa flowers may be automatic or may be effected by insects and other external agents. Untripped flowers form pods and seeds only in rare instances. Automatic tripping is a normal phenomenon. On two plants at Chinook, Mont., in 1909, 33 out of 57 marked flowers became automatically tripped on one plant and set 21 pods, and under the same conditions 64 flowers on the second plant produced 16 pods from 36 automatically tripped flowers. The percentage of pods to flowers on the first plant is 36.84 and on the second 25. These are quite as high as normally occur under natural field conditions. In a similar experiment at Pullman, Wash., in 1910, 60 flowers out of 775 on 9 plants became self-tripped, and 21 of these set pods. In this case only 7.74 per cent of the flowers automati-

cally tripped, of which 35 per cent set pods, while under natural conditions the same season 13.15 per cent of the flowers developed pods.

There is a wide range of variability in alfalfa plants as regards the readiness with which the flowers become tripped, either automatically or by the aid of external objects, and also in their ability to set fruit when tripped. The number of pods set is not proportional to the number of flowers, as a smaller proportion of pods is produced on many-flowered racemes than on few-flowered racemes.

Automatic tripping takes place most frequently in hot sunshine. Humidity is doubtless also a factor. Automatic tripping can readily be observed by focusing a burning glass on open flowers or by simply bringing shaded flowers into the sunshine on a hot day.

Insects are the natural agents of cross-pollination in alfalfa, but even where they are scarce, good crops of seed may be produced. Bumblebees and leaf-cutting bees (Megachile) are the most efficient insects to trip alfalfa flowers. Honeybees secure much honey from alfalfa flowers, but trip only a very small percentage of the blossoms. Night-flying insects are of negligible value. Butterflies have never been observed to trip a flower during the course of these studies.

Rain or wind causes but few alfalfa flowers to become tripped.

Automatic tripping with consequent self-pollination probably results in the setting of as many pods as does tripping by insect visitors, at least in the West. This conclusion is also in accord with the observation that excellent seed crops are produced in sections where bumblebees and other insects capable of tripping alfalfa flowers are decidedly scarce.

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